Resistance to *Pseudomonas solanacearum* in Potato: Specificity and Temperature Sensitivity

E. R. French and Liliam De Lindo

Head, Pathology Department, and research assistant, International Potato Center, Apartado 5969, Lima 100, Peru.
A portion of this research was conducted at, and with the support of, the Southeastern Plant Environment Laboratories phytotron and the Plant Pathology Department of North Carolina State University, Raleigh 27650.
Accepted for publication 16 March 1982.

ABSTRACT

French, E. R., and De Lindo, L. 1982. Resistance to *Pseudomonas solanacearum* in potato: Specificity and temperature sensitivity. Phytopathology 72:1408-1412.

In growth chamber experiments, plants of susceptible and resistant potato cultivars were inoculated with *Pseudomonas solanacearum* by soil infestation without wounding (SI) or by stem puncture (SP) and maintained in warm and cool regimes (28/16 and 20/8 C day/night, respectively). SP and warm treatments each increased susceptibility. Latent infection developed at cool temperatures in cultivars inoculated with SP, but not SI, and at warm temperatures with SI (those inoculated by SP were all rated susceptible). In a factorial experiment with plants of seven potato cultivars and seven *P. solanacearum* strains conducted in a screenhouse under warm conditions, disease ratings were consistent with field results. Resistance derived from *Solanum phureja* has been found to be adequate for the Andean highlands. For warmer climates than those in which potatoes are usually grown, integrated control measures will be needed to control the disease.

Resistance in potato, *Solanum* (Sect. Tuberarium Dun.), to *Pseudomonas solanacearum* E. F. Smith (E. F. Smith) has been sought (22). Some cultivars of *S. tuberosum* subsp. *tuberosum* were found that had considerable bacterial wilt, but gave reasonable yield (15,16). Some cultivars grown commercially in the United States were considered to have moderate resistance (12,17). In the United States, cultivar Katahdin was considered to be more resistant than Red Pontiac and, in Uruguay, Red Pontiac was considered to be more resistant than Kennebec (9). Cultivar Renacimiento (*S. tuberosum* subsp. *andigena*), which has a high level of tolerance, became latently infected in northern Peru and, because it was grown extensively, bacterial wilt became widespread (8). High levels of resistance were found in six clones of *S. phureja* (23). In crosses with subsp. *tuberosum,* resistance was found to be dominant and controlled by only a few genes. The data suggest that three dominant nonlinked genes segregated in one cross. Some deviation from expected ratios suggested that other genes were also involved (18–21). Some selections proved resistant in the field in tropical countries. The Peruvian Potato Program released two selections, Caxamarca in 1976 and Molinera in 1977, from this material (11,20,22).

Screening of seedlings for resistance has been done by inoculation of the bacteria into the stem. Resistance expressed following infestation of the soil without wounding appears to be more representative of field resistance (3,4). Gonzalez et al (10) developed a screening procedure in which a suspension of bacteria was added to flats with 20-day-old seedlings whose roots had been cut. Fewer plants survived with high concentrations of the bacteria in the inoculum, and some surviving plants developed bacterial wilt after being transplanted to pots.

Two *S. phureja* clones and two cultivars selected as resistant to bacterial wilt in the field in tropical America (3) were tested under warm and cool temperature regimes against seven isolates from different parts of the world. The tests were performed in growth chambers at the Southeastern Plant Environment Laboratory phytotron at Raleigh, NC (14). Susceptibility was greatest in the warm regime, and it increased with inoculum concentration and wounding. The specificity of resistance was apparent in some cultivar-isolate combinations, but the small number of plants used was not adequate to support statistically firm conclusions (4).

The purpose of the larger-scale experiments reported here was to test the interaction of the same isolates and cultivars and one additional cultivar in the rather uniform warm environment during the summer near Lima, Peru.

MATERIALS AND METHODS

Potato cultivars were provided by P. R. Rowe and L. Sequeira of the University of Wisconsin. *S. phureja* 1386.15 and 1386.26 had been derived from open-pollinated berries of clone 1386, a selection made by Thurston and Lozano (23). The pedigrees of hybrids V-7, 6-5, T-3, and BR-60.14 and their reaction to *P. solanacearum* in field trials in tropical countries are presented in Table 1. Clone NC 59.B5-1 (NC) was provided by F. Haynes (16). Russett Burbank (RB), a cultivar developed in the United States, was used as a control.

Plants of T-3 were raised from rooted cuttings. Plants of the other cultivars were raised from tubers produced either in the greenhouse or the field. Test plants were grown in a greenhouse at 18 ± 2 C for 30 days in 500-ml plastic pots in equal parts of gravel, sand, and Jiffy mix. They were placed in the growth chambers for 3 days and then inoculated. The temperature regimes were selected to simulate those in which potatoes are grown in the warm, low

TABLE 1. Potato cultivars containing genes for resistance to *Pseudomonas solanacearum,* their pedigrees and known field reactions

Cultivar	Pedigree	Resistance	
		High in:	Low in:
1386.15	*Solanum phureja*	Colombia	Peru
1386.26	*S. phureja*	Peru	···
V-7	PI 214371[a] × 1386.5[b]	Costa Rica Brazil	···
6-5	311.8 (US-W29[c] × 1386) × Katahdin[d]	Costa Rica Peru	Brazil Mauritius
T-3[e]	Chippewa[d] × 1386.26	···	Colombia Peru
BR-60.14	ICA-Purace × 8-14[f] (1386.5[b] × Katahdin)	Peru	···
NC 59.B5-1	*tbr*[d]	···	N. Carolina
Russet Burbank (Control)	*tbr*[d]	···	N. Carolina

[a] United States plant introduction with resistance to virus A & Y.
[b] Bacterial wilt resistant in Peru.
[c] Haploid of *Solanum tuberosum* subsp. *tuberosum (tbr).*
[d] *tbr* as defined under footnote c.
[e] Used only in stem puncture vs. soil infestation inoculation experiment.
[f] Susceptible in Peru.

0031-949X/82/11140805/$03.00/0

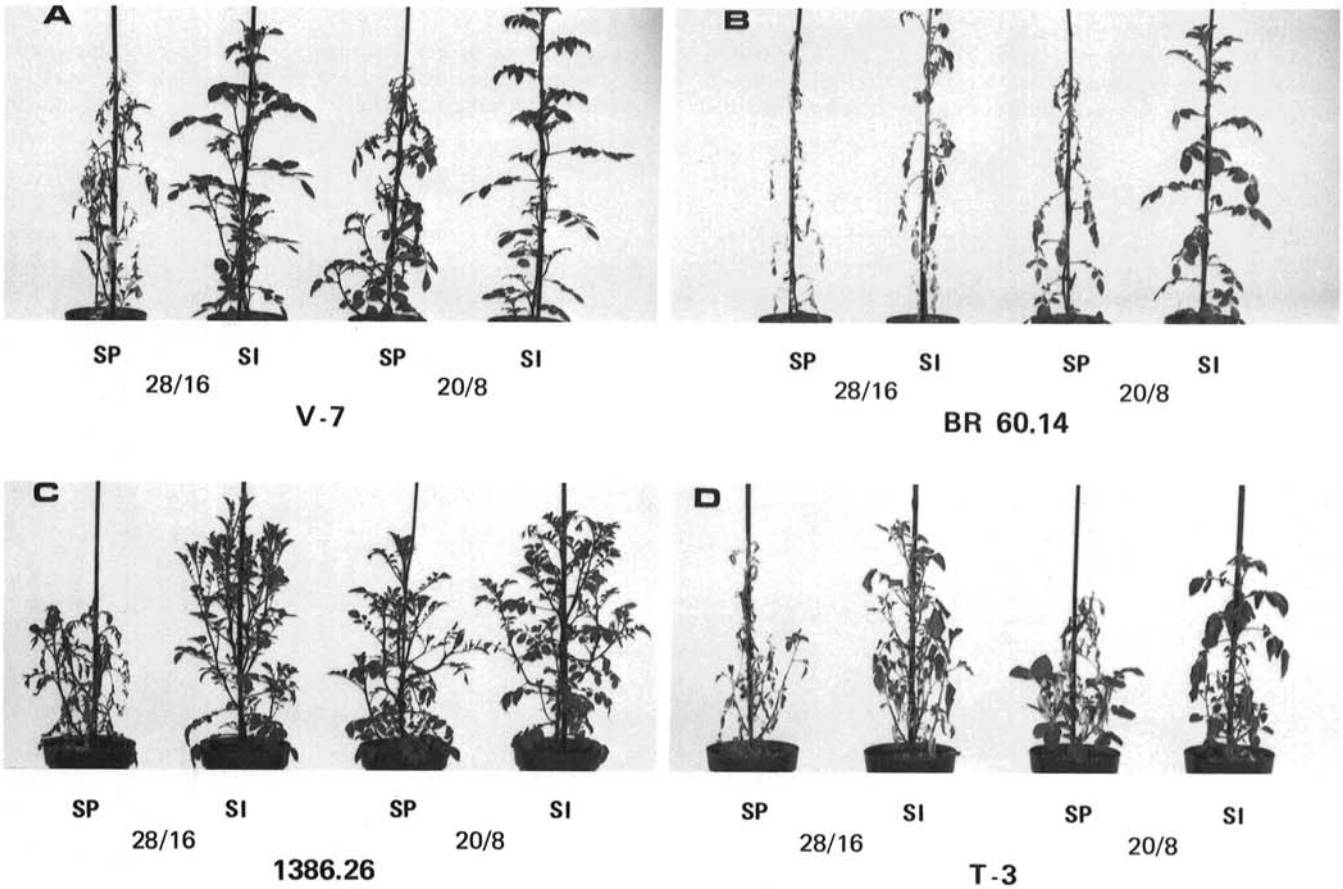

Fig. 1. Symptoms of bacterial wilt in plants of potato cultivars **A,** V-7; **B,** BR 60.14; **C,** 1386.26; and **D,** T-3 after they were inoculated either by stem puncture (SP) or soil infestation (SI) and maintained at warm or cool temperature regimes (day/night) for 22 days.

TABLE 2. *Pseudomonas solanacearum* potato isolates tested, their race/biovar classification, geographical origin, and source

Isolate	Race/biovar	Origin	Source
P1	3/II	Chiguirip, Lambayeque, Peru	L. Nielsen & E. French
013[a]	3/II	Huambos, Cajamaraca, Peru	I. A. Herrera
S207	3/II	Popayan, Colombia	
S213 (048)	3/II	Paraiso, Costa Rica	L. Sequeira
K51 (051)	1/I	Pamlico County, N. Carolina, U.S.A.	L. Sequeira A. Kelman
S245 (049)	1/III	Atherton, Queensland, Australia	L. Sequeira
K197 (047)	1/I	Kenya	A. Kelman
K56 (056)	3/II	Israel	A. Kelman

[a]Strain numbers beginning with 0 are CIP accession numbers.

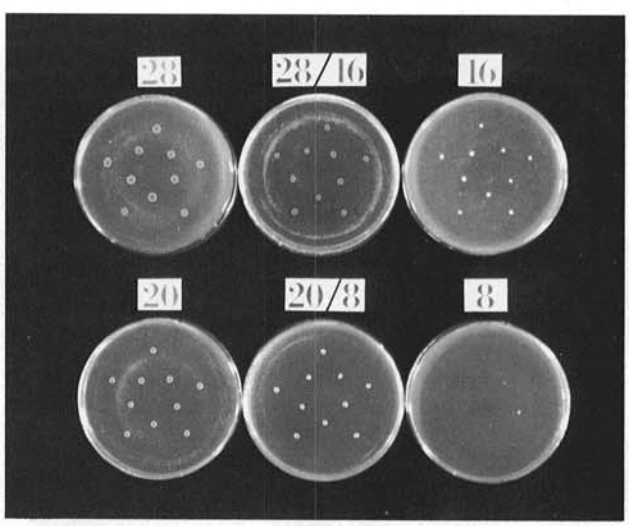

Fig. 2. In vitro growth of strain P1 of *Pseudomonas solanacearum* after 4 days at the temperatures shown. The day/night temperatures (C) of the growth chambers were 28/16 and 20/8.

TABLE 3. Susceptibility (S) and resistance (R) of four potato cultivars inoculated by stem puncture (SP) or by soil infestation (SI) with strain P1 (race 3/Bv II) of *Pseudomonas solanacearum* and incubated for 22 days at two temperature regimes

	Warm (28/16 C)		Cool (20/8 C)	
Cultivars[a]	SP	SI	SP	SI
1386.26	S	R	R	R
V-7	S	R	S	R
BR 60.14	S	S	S	R
T-3	S	S	S	S

[a]Cultivars ranked in order of decreasing resistance.

elevation (1,000 m) and cool, middle elevation (2,400 m) tropics. The warm regime consisted of 12 hr at 28 ± 1 C and 12 hr at 16 ± 1 C (28/16). The cool regime was 20/8 ± 1 C. High light intensity (5,000 lux) was provided at the beginning of the experiments and gradually reduced to ~4,000 lux. Problems with oedema (2) were reduced by having 1 hr of incandescent lighting (30% of the luminosity) prior to the additional illumination with fluorescent lights for 10 hr more each day.

For screenhouse experiments in Lima, plants were grown from seed pieces that had been dipped in 0.3% thiobendazole (Mertect) for 2 min. When main stems were 15–20 cm tall, other stems were severed aseptically at pot-rim level. A complete fertilizer solution was applied weekly. Temperatures during the three summers had average maxima 29.5 ± 2 and minima 16.2 ± 2 C. Daylength was 12.5 hr ± 15 min.

The strains of *P. solanacearum,* their race/biovar (6), geographical origin and source, are given in Table 2. Strain P1 was used in the growth chamber experiments, and a similar strain, 013,

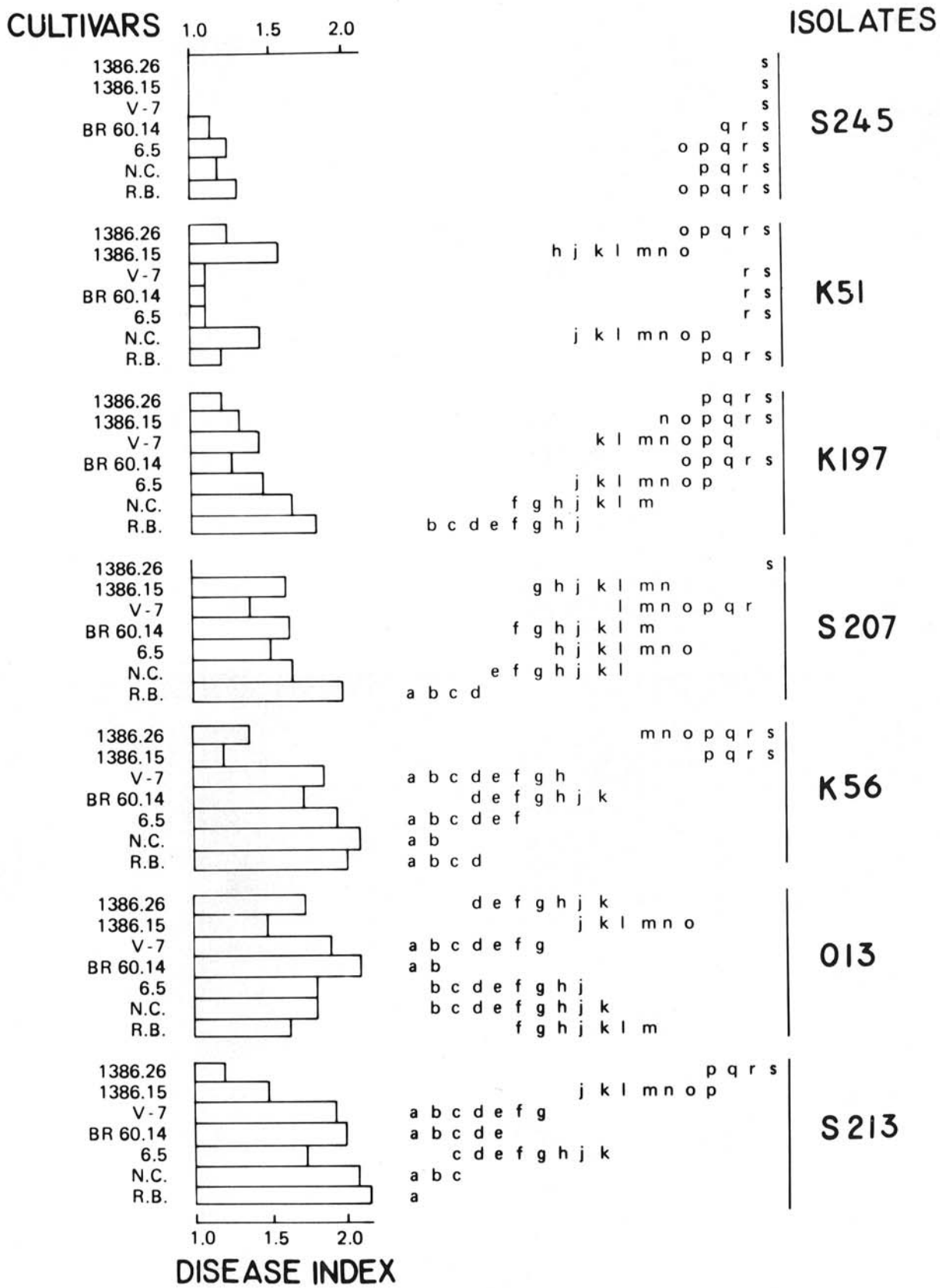

Fig. 3. Disease indices for the interaction of seven potato cultivars and seven strains of *Pseudomonas solanacearum.* Horizontal bars show average readings on a scale ranging from 1 = no symptoms to 5 = complete wilt or plant death. Bars followed by the same letters are not significantly different, $P = 0.01$.

for the screenhouse work. Cultures were purified by selecting wild-type colonies on Kelman's medium (13). Inoculum was grown on the same medium but without tetrazolium salt for 2 days at 30 ± 1 C. For studies of the growth of strain P1 at different temperatures, the stock cultures were previously grown at 28 C for 2 days and then at each of the test temperatures for 4 days. For the two temperature regimes, 28/16 and 20/8 C, a mixture of bacteria from the two different temperature stocks was used as inoculum. Inoculations of petri plates were made with the end of a doubled-back fine wire, in a pattern of 10 equidistant points per plate (three plates per treatment).

Inoculum for the tests of pathogenicity was standardized with a B & L Spectronic 20 colorimeter (± 15%). To help prevent spread of bacteria and soil dessication, saucers were placed beneath each pot, and irrigation water or nutrient solution was added until drainage half filled the saucers.

Disease indices were recorded every third day on a scale of 1 to 5: 1 = no symptoms; 2 = wilt of one leaf; 3 = wilt of up to half the leaves; 4 = wilt of nearly all leaves; and 5 = complete wilt or death.

Inoculations by stem puncture (SP) or soil infestation (SI) were compared in 33-day-old plants, at the warm and cool temperature regimes. Cultivars inoculated were 1386.26, V-7, BR-60.14, and T-3. Stem puncturing was done with a sterile dissecting needle through a drop of bacterial suspension containing 2×10^{10} cells per milliliter in the axil of the third fully expanded leaf from the top. Soil infestation was done by adding 80 ml of a suspension containing 5×10^{10} cells per milliliter to the soil in 1.5-L pots. Each experiment was done twice with six plants per treatment, and with isolate P1.

The testing of differential host range interaction was with a factorial completely randomized experiment with seven cultivars and seven isolates plus a sterile water check (Tables 1 and 2). The experiment was repeated three times with 10 plants per treatment. Each pot containing a one-stemmed 3-day-old plant was inoculated by SI, pouring 50 ml of 2×10^8 bacteria per milliliter into each pot. Disease indices were recorded every three days. Square root transformations were made prior to analysis of variance followed by Duncan's multiple range test.

RESULTS

Stem and root inoculation at warm and cool temperatures. The average disease indices were recorded 14 days after inoculation. These fell into two discrete categories (1.0–2.6 and 3.6–5.0), which were considered as resistant (R) and susceptible (S), respectively, as shown in Table 3. Some cultivars that were resistant by SI in the cool or warm temperature regimes were susceptible by SP, and within a given method of inoculation some that were resistant in the cool regime were susceptible in the warm regime. Isolations from stems yielded *P. solanacearum* from all stem-inoculated plants at both temperature regimes. Reisolation from plants inoculated by SI was possible from susceptible plants (Fig. 1) and from symptomless plants maintained in the 28/16 C regime. Reisolation of bacteria from SI-inoculated plants held at 20/8 C was not achieved.

In vitro growth of *P. solanacearum* at the same temperatures. The comparative multiplication rates of isolate P1 after 4 days is shown in Fig. 2. The greatest growth was at constant 28 C, with less growth at the lower constant temperatures or lower-average dual temperature regimes. No apparent growth took place at 8 C, but colonies developed when these cultures were transferred to 28 C.

Resistance-virulence interaction. Results of the screenhouse inoculations are shown graphically in Fig. 3. Any two bars followed by the same letters are not significantly different, $P = 0.01$. There were some rather major differences between cultivars in their reactions to the seven different strains. For example, cultivars 1386.26, 1386.15, and V-7 remained symptomless when inoculated with strain S245. Cultivar 1386.26 was not infected by S207, but 1386.15 was. Cultivar 1386.15 had the highest disease index rating of all seven cultivars inoculated with strain K 51, but the lowest when inoculated with strain K 56. On the average, as shown by their ranking in Fig. 3, the *S. phureja* hybrids were more susceptible than the two *S. phureja* and more resistant than the two *S. tuberosum* cultivars. The results presented in Fig. 3 clearly show differential reactions between the seven cultivars and the seven strains. The disease ratings for these cultivars were consistant with results obtained in the field (Table 1).

DISCUSSION

A susceptible disease rating was obtained on some SP-inoculated cultivars that were resistant by SI inoculation. The disease ratings on plants inoculated by soil infestation were consistant with those obtained from the seven cultivars in the field with natural infection. It is concluded that inoculation by infesting the soil is a more useful procedure to evaluate for resistance because it more closely correlates to field observations.

The disease severity ratings were higher on all plants at the higher temperature. Whether the effect is on the pathogen or host is not known. It was shown, however, that the pathogen grew more rapidly at the higher temperatures. On the other hand, potatoes are primarily grown in cool climates and the genes for resistance may not be expressed at higher temperature.

No latently infected plants were observed among those inoculated by SI and then incubated at the cooler temperatures. Latent infections did occur when SP inoculated at these cool temperatures and at the higher temperatures by SI inoculation. Therefore, it is possible that resistant cultivars may become infected in infested soil at warmer temperatures than those at which they were originally screened and field tested. These plants would most probably yield infected tubers which, if they did not quickly rot, would, when planted give rise to diseased plants.

The results shown in Fig. 3 clearly show specificity in interaction between the isolates and cultivars studied. The differential reactions are independent of the previous designations of races 1 and 3 (5,18–20,24).

One of the most virulent strains, 013, came from the highlands of Peru. Cultivars Caxamarca and Molinera have been shown to be resistant there wherever grown (11), as also in the highlands of Colombia (G. Granada, *personal communication*). Resistance selected in Taiwan (1) is also being tested in Peru. Resistance seems to be easily transferred (20), but, since resistance is temperature sensitive, it may still be necessary to use integrated control measures (7), particularly for warmer climates.

LITERATURE CITED

1. Asian Vegetable Research and Development Center. 1978. Bacterial wilt resistance studies in white potato. About AVRDC. (The Asian Vegetable Research and Development Center, Shanhua, Taiwan) 2:7.
2. Balge, R. J., Struckmeyer, B. E., and Beck, G. E. 1969. Occurrence, severity and nature of oedema in *Pelargonium hortorum* Ait. J. Am. Soc. Hortic. Sci. 94:181-183.
3. French, E. R. 1972. Evaluación de la resistencia de clones de papa a *Pseudomonas solanacearum*. (Abstr.) Phytopathology 62:757-758.
4. French, E. R. 1972. Progress and problems in selecting resistance to bacterial wilt. Pages 212–214 in: Prospects for the potato in the developing world. E. R. French, ed. International Potato Center, Lima, Peru.
5. French, E. R. 1978. Pathotypes of *Pseudomonas solanacearum* relative to breeding for resistance in potatoes. (Abstr.) Proc. III Int. Congr. Plant Pathol., Munich, W. Germany. p. 290.
6. French, E. R. 1979. Classification, distribution and origin of *Pseudomonas solanacearum*. Pages 28–35 in: Developments in Control of Potato Bacterial Diseases. International Potato Center, Lima, Peru.
7. French, E. R. 1979. Progress in the integrated control of bacterial wilt. Pages 72–81 in: Developments in Control of Potato Bacterial Diseases. International Potato Center, Lima, Peru.
8. French, E. R. Torres, H., Ames de Icochea, T., Salazar, L., Fribourg, C., Fernandez, E. N., Martin, A., Franco, J., Maria de Scurrah, M., Herrera, I. A., Vise, C., Lazo, L., and Hidalgo, O. 1972. Enfermedades de la papa en el Perú. Min. Agric. Bol. Tecn. 77. Lima, Peru. 36 pp.
9. Garcia, S. 1976. Status of potato brown rot in Uruguay. Pages 78–83 in: Planning conference and workshop on the ecology and control of bacterial wilt caused by *Pseudomonas solanacearum*. L. Sequeira and A. Kelman, eds. N.C. State University, Raleigh.

10. Gonzalez, L. C., Sequeira, L., and Rowe, P. R. 1973. A root inoculation technique to screen potato seedlings for resistance to *Pseudomonas solanacearum*. Am. Potato J. 50:96-104.
11. Herrera, I. A., De La Puente, F., and French, E. R. 1978. Variedades de papa peruanas resistentes a *Pseudomonas solanacearum*. (Abstr.) Phytopathol. News 12:264.
12. Jaworsky, C. A., Webb, R. E., Goth, R. W., and Phatak, S. C. 1979. Resistance of potato cultivars to bacterial wilt. (Abstr.) Am. Potato J. 56:467.
13. Kelman, A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. Phytopathology 44:693-695.
14. Kramer, P. J., Hellmers, H., and Downs, R. J. 1970. SEPEL: New phytotrons for environmental research. BioScience 20:1201-1208.
15. Lallmahomed, G. M. 1976. Strains of *Pseudomonas solanacearum* and current research on bacterial wilt in Mauritius. Page 104 in: Planning Conference and Workshop on the Ecology and Control of Bacterial Wilt caused by *Pseudomonas solanacearum*. L. Sequeira and A. Kelman, eds. N.C. State University, Raleigh.
16. Nielsen, L. W., and Haynes, F. L., Jr. 1960. Resistance in *Solanum tuberosum* to *Pseudomonas solanacearum*. Am. Potato J. 37:260-267.
17. O'Brien, M., and Rich, A. E. 1976. Potato diseases. U.S. Dep. Agric., Agric. Handb. 474. U.S. Govt. Printing Office, Washington, DC. 79 pp.
18. Rowe, P. R., and Sequeira, L. 1970. Inheritance of resistance to *Pseudomonas solanacearum* in *Solanum phureja*. Phytopathology 60:1499-1501.
19. Rowe, P. R., Sequeira, L., and Gonzalez, L. C. 1972. Additional genes for resistance to *Pseudomonas solanacearum* in *Solanum phureja*. Phytopathology 62:1092-1094.
20. Sequeira, L. 1979. Development of resistance to bacterial wilt derived from *Solanum phureja*. Pages 55–62 in: Developments in control of potato bacterial diseases. International Potato Center, Lima, Peru.
21. Sequeira, L., and Rowe, P. R. 1969. Selection and utilization of *Solanum phureja* clones with high resistance to different strains of *Pseudomonas solanacearum*. Am. Potato J. 46:451-462.
22. Thurston, H. D. 1976. Resistance to bacterial wilt (*Pseudomonas solanacearum*). Pages 58–62 in: Planning Conference and Workshop on the Ecology and Control of Bacterial Wilt Caused by *Pseudomonas solanacearum*. L. Sequeira and A. Kelman, eds. N.C. State University, Raleigh.
23. Thurston, H. D., and Lozano, J. C. 1968. Resistance to bacterial wilt of potatoes in Colombian clones of *Solanum phureja*. Am. Potato J. 45:51-55.
24. Tschanz, A. T. 1978. Incorporation of resistance to *Phytophthora infestans* and *Pseudomonas solanacearum* into the Andigena potato and the interrelationship of *Meloidogyne incognita* and *Pseudomonas solanacearum* on potatoes resistant to *P. solanacearum*. Ph.D. thesis, Cornell Univ. 131 pp.